

Maternal Antibody Titer Against Avian Influenza Transferred from Hens to The Eggs and Ducklings

Ismoyowati^{1)*}, M Mufti¹⁾, M Samsi²⁾ and A Susanto³⁾

¹⁾Laboratory of Poultry Production, ²⁾Laboratory of Animal Veterinary, ³⁾Laboratory of Animal Breeding, Faculty of Animal Science, Jenderal Soedirman University, Jl. Dr. Suparno 60, Purwokerto 53123, Central Java, Indonesia
Corresponding author email: moy.moyowati@gmail.com; Telephone: 08156704686; Fax: +62-0281-638792

Abstract. This research aimed to evaluate the effectiveness of H5N1 Avian Influenza vaccination in different duck breeds and to assess the magnitude of maternal antibody titer transferred from the vaccinated hen to the eggs and off ducklings as a protector agent against H5N1. Experimental research was conducted on 16 male and 48 female mallards and 16 male and 48 female Muscovy ducks aged 16 week old. The study showed that the vaccination was 83.33 % successful in Muscovy ducks and 100% in in mallards. Result of variance analysis demonstrated that breed and sex did not significantly affected AI antibody titer. AI maternal antibody transferred from Muscovy hens to egg yolks and to off springs was 66.37% and 39.51%, respectively. Female Mallards transferred higher antibody to egg yolks than to off springs (96.40% versus 63.18%, respectively. Antibody titer against AI vaccination was determined through ELISA. This study concluded that AI H5N1 vaccination increased antibody titer in ducks which is transferrable to the eggs produced and ducklings.

Key words: Vaccination, H5N1 virus, antibody titer, egg yolk, ducklings

Abstrak. Penelitian ini bertujuan untuk mengevaluasi efektifitas vaksinasi avian influenza H5N1 yang dilakukan pada bangsa itik yang berbeda. Penelitian juga bertujuan untuk mengetahui besarnya transfer maternal titer antibody dari induk yang divaksinasi ke telur dan anak itik yang dihasilkan untuk perlindungan terhadap infeksi virus H5N1. Penelitian dilakukan secara eksperimental dengan materi menggunakan itik yang terdiri dari itik Tegal jantan 16 ekor dan betina 48 ekor serta entok jantan 16 ekor dan betina 48 ekor umur 16 minggu. Hasil vaksinasi menunjukkan keberhasilan vaksinasi sebesar 83.33% pada entok dan pada itik 100%. Hasil analisis variansi menunjukkan bangsa itik dan jenis kelamin tidak berpengaruh nyata ($P < 0.05$) terhadap titer antibodi flu burung (H5N1). Besarnya transfer maternal antibodi flu burung dari induk entok ke kuning telur sebesar 66,37%, sedangkan pada anaknya 39,51%. Induk itik memberikan antibodi lebih tinggi pada kuning telurnya yaitu sebesar 96,40% dan pada anaknya 63,18%. Kesimpulan dari penelitian ini adalah vaksinasi flu burung H5N1 meningkatkan titer antibodi pada itik yang dapat ditransfer pada telur dan keturunannya.

Kata kunci: Vaksinasi, virus H5N1, titer antibodi, kuning telur, anak itik

Introduction

Waterfowls more specifically ducks are contributing significantly to protein supply of meats and eggs in Indonesia. Duck eggs are mostly produced by native ducks (mallard) while meats are from obtained male mallards, culled mallard hens and Muscovy. Native ducks, one of local alternative fowls, is quite potential, profitable, and reliable source of family income with a stabilized market (Balitbang Deptan, 2010). Ducks supplied national 265.789 ton meat and 29.180 ton egg demand in 2011.

Ducks population in Indonesia is annually increasing with 7.54% growth per annum from 2007 to 2011 (Directorate General of Animal Farming and Veterinary, 2011). Duck farming in Indonesia however is mostly run under traditionally with scavenging system; which from health aspect viewpoint is suspected to promote H5N1 virus distribution (Rainat, 2005; Songserm et al., 2006).

Avian Infuenza is fowl transmissible and deadly disease to human. Avian Influenza in fowls is spreading and infecting in such speed that mortality rate reaches 100%. The past 10

years in Asia people have witnessed the distribution of high pathogen avian influenza (HPAI) virus of H5N1 and H9N2 in wild birds mainly waterfowls. Low pathogen avian influenza virus (LPAI) is continuously spreading among domestic fowls, specifically those under direct or indirect exposure to wild birds. H5 and H7 subtype viruses have undergone mutation into malignant virus, HPAI. Avian Influenza Virus infected several types of birds including the free birds, confined domestic fowls such as ducks, chickens, turkeys and other fowls (Capua and Alexander, 2009). Avian Influenza has brought significant economic loss in both farming industry and traditional farm, staggering billion rupiahs within 2012 and 2013. The common AI control strategy on fowls is extermination of infected fowls to certain perimeter (stamping out/preemptive culling), biosecurity and vaccination. Vaccination has become one of the main strategy to overcome AI in Indonesia and several countries (OIE, 2012).

Fowl epidemic risk has long been concerned in poultry industry since the highly pathogenic Avian Influenza Virus H5N1 occurred in Hong Kong during 1996 and 1997. Within 2003 and 2009 H5N1 virus has spread in domestic and wild fowls in 62 countries in Asia, Europe, Africa, and Middle East (WHO, 2009). In January 2009 it was recorded 403 AI contagion cases (HPAI) responsible to 254 death in Asia, Middle East and Africa (World Health Organization, 2009). Integrated project is subject to overcoming control over AI distribution by developing diagnostic test and vaccine, particularly technology, strategy and supervision to prevent the ever spreading AI (Cardona et al., 2009).

This research aimed to evaluate the effectiveness of H5N1 vaccination in different duck breeds and to assess the extent of maternal antibody titer transfer from the vaccinated hens to the egg and ducklings.

Materials and Methods

The research materials were obtained from duck farming in East Java which were composed of 16 male and 48 female mallards and 16 male and 48 female muscovy aged 16 week old, making up 128 heads altogether. Ducks were reared in groups with 1:3 of sex ratio. Feed in production period (layers) consisted of 35% yellow cornmeal, 40% rice bran and 25% duck concentrate with feed nutrient of 17% protein, 2900 kcal/kg metabolic energy, 3.02% Calcium and 1.06% phosphor. The materials used in the study were H5N1 ELISA kits, Red Blood Cells (RBC) 0.5%, physiological NaCl, Alcohol 70%, Pusvetma AI antigen, and ducks serum.

First experiment aimed to recognize body immune difference between mallards and muscovy ducks against Avian Influenza by Completely Randomized Design method (AI pre-vaccination) and AI post-vaccination, making up 8 treatment combinations. Each experiment consisted of one male fowl and three females having four replications. The treatments were as follows: (1) non AI vaccinated male mallards (control); (2) non AI vaccinated female mallards (control); (3) non AI vaccinated male muscovy (control); (4) non AI vaccinated female muscovy (control); (5) AI vaccinated male mallards; (6) AI vaccinated female mallards; (7) AI vaccinated male muscovy, and (8) AI vaccinated female Muscovy.

AI vaccine administered in this research was produced by Pusat Veteriner Masyarakat-PUSVETMA (Public Centre of Veterinary) Indonesia. AI vaccination was given at 16 week old ducks and blood sample was taken from all ducks on the 21st day post-infection (vaccination) for antibody titer against AI using HI (Haemagglutination Inhibition) test. Serum obtained was inactivated by 30 minute heating at 56°C before HI test (OIE, 2012).

HA test was performed to assess the AI antigen titer which is going to be used in HI

test. HA was perfect (100%) if hemagglutination is clearly visible as red blood cells layer evenly spread on the bottom hole and clear liquid is observed on the surface without dotted sedimented red blood cells in the middle of the hole. On HI test, 4 HA unit antigen/0.025 ml was needed warranted through retitration.

Hemagglutination Inhibition procedure was as follows: 0.025 mL physiological NaCl was pipetted into well 1 through well 12 of microplate. As much as 0.025 mL of Mallard blood serum was added into well 1 and then was mixed using micropipette. Serial dilution was performed by moving 0.025mL micropipette from well 1 to well 2, then mixed and moved to well 3, and so forth through well 10. Well 11 did not get serum because it was the red blood cell control, while well 12 got the serum as serum control. Then 0.025 mL 4 HA (hemagglutination) was pipetted to well 1 through 10. Well 1 through 12 was added with 0.05mL RBC 0.5% and the microplate was slowly shaken and left to read the red blood cell control. Antigen used for serologic test was Ag H5N1. Perfect HI reaction (100%) was marked by RBC spot-like sediment at the well bottom. The obtained data were subject to tabulation and analysis of variance. Honestly Significant Difference Test (HSD) was performed to assess the significant effect of the treatments (Steel and Torie, 1995).

The second experiment observed the extent of maternal antibody transferred to egg (egg yolk) and offsprings. Fifty percent eggs laid from each mating group were sampled for antibody titer observation on AI in egg yolk and the other 50% was hatched. After reared for four weeks, the ducklings had their blood sample taken to observe the antibody titer against AI and the hen's blood were sampled at 26 week old.

Egg antibody titer (IgY) was determined according to the previously enacted procedure

by Hamal et al. (2006). Eggs were cracked to separate the white from the yolk then they were put into Petri cup. The yolk membrane was washed with aquadest. The yolk was then put into tube to measure the volume, added with Dulbecco twice the egg volume, stirred to homogenize, and added with chloroform as much as the egg volume. The suspension was stirred to homogenize and emulsify, then centrifuged at 1000×g for 30 minutes at room temperature. The centrifugation separated emulsion into three distinct layers, orange solution (lecithin) at the bottom, half yolk solid in chloroform in the middle, and antibody-contained protein serum above was kept at -20°C until analysis.

The amount of antibody titer against AI was determined using egg yolk and blood serum sample following the procedure of ELISA method (Enzyme-linked immunoassay) (OIE, 2012). The main principle of ELISA method is to utilize indicator enzyme for immunological reactions. Antigen is bound at the first polystyrene micro titer plate. Antiserum containing anti-peptide antibody then was added into the wells. The second antibody which is specific for the first antibody was labeled for detection and was added into the wells. The second antibody is an enzyme and this enzyme is responsible for catalyzing colour formation of the substance. The substance colour then was measured and number of the antibody can be counted (Bioon, 2010). The value of transferred antibody from hen to the offsprings was obtained from dividing the ducklings' antibody titer of egg yolk and serum by hens' antibody titer then multiplied by 100.

Results and Discussion

The Difference of Antibody Titer Against Avian Influenza (AI) In Ducks Between pre- And Post-Vaccination

Result demonstrated that vaccination on mallards and muscovy hens was 16.67% failure

Table 1. Avian influenza antibody titers pre- and post-vaccination in ducks and Muscovys using HI test (\log_2^4)

Water fowls	Pre-vaccination	Post-vaccination
Male Muscovy	0.25±0.707 ^a	2.00±2.733 ^b
Female Muscovy	1.75±1.669 ^a	4.539±5.561 ^c
Male Mallard	0.50±1.414 ^a	3.385±4.350 ^b
Female Mallard	2.00±2.828 ^a	5.278±7.741 ^c

Values bearing different superscript at the same row and different column shows highly significant difference at HSD test

in muscovy and 100% successful in mallards. Analysis of variance showed that duck breed did not significantly affect ($P < 0.05$) the antibody titer against AI. AI vaccination showed that antibody titer increases between inactive AI vaccinated and non-vaccinated ducks. The vaccinated waterfowls showed antibody titer on the 21st day of post-vaccination. Female ducks had antibody titer higher than male ducks at post-AI vaccination (Table 1).

Antibody in serum culminated after 10-14 days before the sudden drop (Tizard, 2013). The indication of successful vaccination was by measuring antibody titer in which positive HI titer was marked with inhibition diluted at 1:16 (2^4) or \log_2^4 using 4 HAU antigen (OIE, 2012). The higher antibody titer in HI test, the higher immunity against the right antigen was although this is not absolute (Allan et.al., 1978).

This result was supported by Hidayanto et al. (2010) reporting challenge test on Specific Pathogen-Free (SPF) that the less vaccine dose given, the higher conversion percentage of the HI antibody titer was. The post-challenge antibody titer was higher in 3 of 5 serum sample (60%) of chicken vaccinated with I/625 dosage. At 1/125 dosage, 4 of 14 serum sample (29%) of post-challenged antibody titer increased, however at 1 dosage the increase was absent. Other research on non-vaccinated fowls' serum sample taken from traditional farming in Banten reported that AI H5N1 had infected 42.5% chickens, 88.8% muscovy, 100% geese, 100% mallards, 50% turtledoves and 8.3% pigeon (Kurniadhi, 2006). Vaccination creates humoral antibody response and

intensity of the antibody response was varied among bird species. Immune response against neuraminidase protein contribute to protection, however immune response against viral internal protein is generally unprotective (Swayne and Kapczynski, 2008).

Maternal Antibody Transfer on Ducklings

Antibody titer measurement on eggs and the offsprings of AI-vaccinated hens showed decreasing percentage of maternal antibody against AI transferred from hens to eggs then deposited to the offsprings. Antibody titer serum averaged 52.89-96.85 mg/ml in muscovy hen; 32.58-93.48 mg/ml in egg yolk, and 4.41-76.25 mg/ml in muscovy ducklings. The low antibody titer against AI in duck hens, eggs, and the offsprings was because the vaccination was done at 16 weeks of age and the vaccination was not repeated at 26 weeks of age. Figure 1 and 2 showed the percentage of muscovy's maternal antibody transfer to the eggs or IgY and to offsprings as much as 66.37% and 39.51%, respectively. Antibody transferred from mallard hens to the egg yolk was more than to the offsprings (96.40% vs 63.18%).

AI vaccinated hens produce offsprings with specific maternal AI antibody. High maternal antibody titer was needed for clinical protection and post-infection virus titer reduction in fowls, while low antibody titer would interrupt vaccine effectiveness (Maas et al., 2011). Fowls transfer maternal antibody to the offsprings by depositing antibody into the eggs (IgY) (Brambell, 1970). In fowls, IgY maternal transfer to offsprings is through two steps. The first step, IgY was deposited to the

egg yolk by IgY receptor in follicle in ovary through the hen's bloodstream (Cutting and Roth, 1973; Loeken and Roth, 1983). In the second step, IgY was transferred from egg yolk to the offsprings through embryo's blood circulation. Kramer and Cho (1970) reported that yellow IgY was transferred from low level in yolk sac into the embryo's blood circulation at early period (7 days old). Level of maternal antibody transfer started to increase at 14, 19 days to hatch and IgY transfer rate sharply increased from egg yolk to the embryo's blood circulation (Kowalczyk et al., 1985). The amount of IgY transferred to the egg yolk was reported to equal with the IgY concentration of hen serum (Loekendan Roth, 1983; Al-Natour et al., 2004).

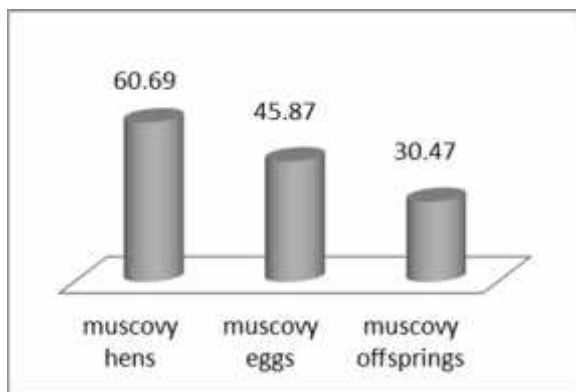


Figure 1. Percentage transfer of maternal antibody titer against AI from muscovy hens to egg yolk and offsprings.

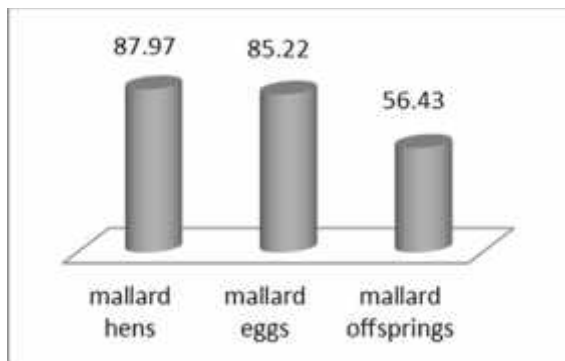


Figure 2. Percentage of maternal antibody titer against AI transferred from hens to the eggs and offsprings.

Fowls in early period were highly prone to pathogenic microbe due to the partly developed body immune system at the first few weeks. Maternal antibodies are the prime device of specific antigen protection. Several researches had reported specific maternal antibody transfer to the offsprings through eggs and its function to protect the newly hatched ducklings from pathogenic microbe (Sharma et al., 1989; Heller et al., 1990; Mondal and Naqi, 2001; Sahin et al., 2001; Rahman et al., 2002; Ahmed and Akhter, 2003). Most chicks with high maternal immunity had high virus titer in trachea within four days after H5N1 infection. Low virus titer was only found in cloaca in most chicks group (Maas et al., 2011). The newly-hatched ducklings synthesize endogen antibody according to the type of antibody (Hamal et al., 2006). Lawrence et al. (1981) stated that cell B IgY secretion was not detected at the newly-hatched chicks. Hamal et al. (2006) reported a direct consistent relation between antibody titer and specific antigen in egg yolk transferred from hens to the offsprings namely for anti-NDV and anti-IBV antibody, ranging from 31 to 41%. Estimation of maternal antibody titer half-life respectively was 5.3, 4.2, 7.0, 5.1, 3.9, 3.8, 4.9, 4.1, 6.3, and 4.7 days for avian encephalomyelitis (AEV), avian influenza virus (AIV), chicken anemia virus (CAV), infectious bursal disease virus (IBDV), infectious bronchitis virus (IBV), infectious laryngotracheitis virus (ILT), Mycoplasma gallisepticum (MG), Mycoplasma synoviae (MS), and reovirus (Reo) (Gharaibeh and Mahmoud, 2013).

Conclusions

It can be concluded that AI H5N1 vaccination was effective to increase duck's antibody titer being more successful rate in female than in male ducks. Duck eggs and ducklings had maternal antibody transferred from the AI H5N1 vaccinated hen. Prevention and control of avian influenza in ducks can be done using the

integrated vaccination program in accordance with the vaccine strain of the avian influenza virus in the farm area.

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Statement of animal right: This study was conducted with pay attention to the ethical standards animal. Ducks kept in a cage that meets the convenience aspect animal. Methods of vaccination and blood sampling carried out in accordance with animal health procedures.

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